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# TB123: Experimental Application of B.t.i. for Larval Black Fly Control: Persistence and Downstream Carry, Efficacy, Impact on Non-target Invertebrates and Fish Feeding

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for Larval Black Fly Control:  
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MAINE AGRICULTURAL EXPERIMENT STATION  
UNIVERSITY OF MAINE



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## INTRODUCTION

Biting and swarming black flies can cause serious discomfort to humans in Maine. In spite of this problem, the advisability and means of reducing their numbers remain controversial. Control is generally aimed at the larvae in streams. Environmentalists are concerned with the persistence of control materials in streams used as drinking water and for recreation, and that control agents aimed at black fly larvae will affect non-target forms in the stream. Historically, these concerns have made serious consideration of black fly control measures in Maine unacceptable.

The most promising control agent available at present is the biological insecticide *Bacillus thuringiensis* var. *israeliensis* de Barjac (*B.t.i.*). This material and its use in the field have been reviewed (3, 4 and 6). It is a spore forming bacterium which, at sporulation, produces crystals of toxic protein which are lethal to larvae of mosquitoes and black flies when ingested. *B.t.i.* does not normally cause infectious disease which spreads from insect to insect through transmission of spores. The insecticidal crystalline toxin acts as a stomach poison and has no contact effect. The crystal is a protoxin which is broken down in the midgut of susceptible larvae into toxic sub-components. This conversion seems to occur only in mosquitoes, black flies and some related Diptera and so to account for the host specificity of the material. Laboratory and field studies have shown that a wide range of non-target organisms occurring with black flies and mosquitoes are not susceptible to *B.t.i.* or are susceptible only at dosages greatly in excess of those used in control operations. Only Diptera closely related to black flies such as some Chironomidae and Blepharoceridae (1,3 and 4) have been shown to be susceptible. Increased drift in Ephemeroptera and Trichoptera was shown in one study (5), but other reports showed no effect on these insects.

Various factors may influence the insecticidal activity against black fly larvae. Laboratory studies have demonstrated the effects of temperature, larval age, species of black fly, concentration of inoculum and formulation on insecticidal activity (4). Additional factors, such as stream discharge and profile (especially depth-to-width ratio), turbidity, turbulence, density of aquatic vegetation and filter feeding organisms, dilution of *B.t.i.* and duration of treatment may influence larvicidal activity under field conditions.

In the summer of 1985 a field experiment was conducted in the Sugarloaf area of Maine on the use of *B.t.i.* to reduce the numbers of black fly larvae in the Carrabassett River and a tributary stream. The objectives were to determine the rate of application necessary to produce an acceptable reduction in black fly larvae, to study the fate and persistence of *B.t.i.* in a stream following application, to determine the impact of *B.t.i.* on the abundance and drift of non-target stream insects and on the feeding success and diet composition of fishes in the treated streams.

## STUDY AREA

The study area was the Carrabassett River in the area of the golf course at Sugarloaf, Maine and a tributary here designated as No Name Stream which flows east under Rte. 27 at Bigelow before joining the Carrabassett. The application site on the Carrabassett River was near the upstream boundary of the golf course just above the upper bridge. The stretch of the river above the application site is referred to as the control area and that below as the treated area. Sampling stations were established at intervals downstream from the application site. The same procedure was followed on No Name Stream.

The black fly fauna of the study area was surveyed in 1984 (K. E. Gibbs and K. R. Hardy, unpublished report to the Sugarloaf Mountain Corporation). Black fly larvae were abundant in the Carrabasset River and No Name Stream, and the species causing the greatest human nuisance by biting and swarming were *Prosimulium fontanum* Syme and Davies, *P. mixtum* Syme and Davies, *Simulium venustum* Say, *S. corbis* Twinn, *S. parnassum* Malloch, *S. tuberosum* (Lundstr.) and *Stegopterna mutata* (Malloch).

## METHODS AND MATERIALS

### *B.t.i. and Application Procedures*

The formulation of *B.t.i.* used was the aqueous suspension, Vectobac® (AS)-14 manufactured by Abbott Laboratories. Four experimental applications were made during the summer as shown in Table 1.

Application was by direct metered introduction into the stream and followed the method of D. P. Molloy, New York Museum and Science Service, New York State Education Department, Albany, New York, 12230 (personal communication).

### *Fate and Persistence of B.t.i.*

It should be noted that, though spores/ml of water may not be directly and consistently related to toxicity, their use in discussing movement of treatment suspensions is valid. The intent of this study was to examine the distribution of *B.t.i.* spores as a reference to the distribution of the spore crystals. This experimental application was made on August 5 when the discharge of the river was 0.15 m<sup>3</sup>/sec. Rhodamine-B dye was used to determine the time taken for transportation of material from the application site to the sampling stations. *B.t.i.* was applied at the rate of 10 ppm for 5 min, 2 min after the dye was applied. The concentration of spores passing through sampling stations at 150 m, 750 m and 1250 m was monitored starting at 2 min after the dye reached each station. Water samples were taken at 3 min intervals for 30 min and then at 5 min intervals for the next 30 min.

Concentrations of *B.t.i.* spores in water at the sampling sites were determined following the method of Frommer (2). Pre- and post-treatment water samples were collected in 125 ml containers by submerging the containers midstream at a medium water depth for each time-distance. Water samples were kept refrigerated until processed in the laboratory. On the day plate counts were prepared, a 10 ml aliquot of each sample was removed from the water bottle after the bottle was shaken vigorously by hand. Each 10 ml aliquot was heat shocked in a water bath for 30 min at 60°C (prior to preparation of the dilutions to be plated). Samples were heat shocked to eliminate background vegetative stage bacterial and fungal contaminants. Sterile distilled water blanks were used to prepare serial dilutions of the samples. Initially, tenfold dilutions were used but later fivefold dilutions were used. Dilutions were prepared using Corning plastic disposable pipettes, then thoroughly mixed with a Vortex mixer. One ml of each dilution to be plated was added to a 100 x 15 ml petri dish and slowly swirled to spread the spores. Approximately 15 ml of molten Difco® tryptose blood agar base was added to each plate. Agar was maintained liquid throughout the procedure. Solidified agar plates were incubated for 15-17 h at room temperature.

The number of colonies resulting was counted using a Fisher colony counter. The dilutions selected to determine the concentration (i.e., the number of colony forming units per ml) were represented by the plates containing between 30 and 300 colony forming units. Three plate replications were prepared for estimating mean spores/ml for each time and distance.

#### *Impact on Invertebrates*

The impact of *B.t.i.* applications on black fly larvae attached to the substrate and in the drift, and on non-target invertebrates in the substrate and in the drift, was measured.

*Percent Mortality of Black Fly Larvae.* Percent mortality of black fly larvae was calculated by counting the numbers of living and dead larvae on natural rock substrates at downstream treated and upstream control sampling stations following *B.t.i.* application. Attempts to use artificial substrates in the form of plastic streamers were abandoned as the streamers were only lightly and irregularly colonized. Counts were made 2-6 h, and on one occasion 24 h, after application. Living and dead larvae were identified to species.

*Drift.* Duplicate 15 min drift samples were taken in the control area and in the treated area 225 m below the application site in the Carrabassett River during the June 17 and July 11 treatments. Similar procedures were followed for the July 11 application in No Name Stream except that the nets in the treated area were 50 m below the treatment site. The nets used were 16 cm high, 34 cm wide and 1 m long, with a mesh aperture of 350  $\mu$ m. All material in the

drift was immediately preserved in 95% ethyl alcohol. The volume of water passing through the net during the sampling period was determined by measuring the flow rate (m/s) and the area of the net submerged ( $m^2$ ). The numbers of organisms drifting were calculated in numbers per unit volume of water ( $n/m^3$ ) in order to reduce the variability caused by variable flow. The final numbers presented represent means of the duplicate samples or, in a few cases where one was lost, only one sample.

*Substrate Samples.* To determine if applications of *B.t.i.* caused changes in the standing stock (numbers) of non-target macroinvertebrates (mainly aquatic insects) in the stream bottom, artificial substrates were placed in the stream bottom three weeks before the sampling date to allow time for colonization. The substrates consisted of "rock bags" or plastic mesh bags (grocery store fruit bags) filled with 2 kg of rocks having a maximum diameter of 5-6 cm. Five of these bags were removed from the control and treated areas of the stream pre- and post-treatment with reference to the June 17 and the July 11 applications to the Carrabassett River and No Name Stream, respectively. The organisms were washed from the rocks and those retained by a #40 sieve were preserved in 80% ethyl alcohol and returned to the laboratory for sorting and identification.

#### *Impact on Fish Feeding*

Only brook trout (*Salvelinus fontinalis* (Mitchill)) and slimy sculpins (*Cottus cognatus* Richardson) were common in reaches of the Carrabassett River and No Name Stream included in the study. Thus, only these two species were collected for analysis.

*Fish Sampling and Gut Content Analysis.* Fish were sampled from control and treated areas, pre- and posttreatment, from the Carrabassett River with reference to the June 17 application and from No Name Stream with reference to the July 11 application. Fish were collected by electro-shocking, and an attempt was made to obtain 10 fish of each species from each area on each date. All fish were immediately preserved in 80% ethyl alcohol, and the larger fish were slit along the abdomen to allow penetration of the preservative. In the laboratory, weight and total length of the fish were determined and the fish stomach and intestine were removed. The volume of the gut contents was determined by volumetric displacement in the barrel of either a 1 or 3 ml syringe. The gut content was then placed on a grid under 10x magnification and separated into the following categories: Simuliidae (black fly) larvae, Blepharoceridae larvae, other Diptera (mainly Chironomidae) larvae, Diptera pupae, Ephemeroptera (mayfly) nymphs, Plecoptera (stonefly) nymphs, Trichoptera (caddisfly) larvae, terrestrials (insects and spiders which had fallen into or onto the water

from the terrestrial environment), miscellaneous (unidentified material including organic and inorganic material such as leaves and sand ingested incidently with the food material). Black fly larvae are distinctive because of the well sclerotized head capsule and cephalic fans and could be easily identified. The number of black fly larvae in each gut was recorded as was the number of fish in the sample containing black fly larvae. The percent of the total gut content represented by black fly larvae was determined by counting the number of squares of the grid covered by black fly larvae divided by the total number of squares covered by all the material present. The number of fish in which they occurred and the percent of total gut content were determined for the other food categories.

## RESULTS

### *Fate and Persistence of B.t.i.*

Pretreatment background spore counts were at insignificant levels in stream water and thus did not interfere with the analysis of samples collected post-treatment. The dye reached 150 m in 8 min, 750 m in 41 min and 1250 m in 1 h and 33 min. Water sampling was initiated 2 min later. The distribution of spores as they moved through the down stream collecting stations is shown in Fig. 1. Results at the three sites differ in time to peak concentration, peak concentration attained, concentration spread in relation to the 5 min application time and rates of ascent and descent of spore concentrations following the initiation and termination of application. At the 150 m site the time to peak concentration was 6 min, at 750 m 15 min, and at 1250 m 15 min (the only time at which a spore count over the minimum level of detection was recorded). At 150 m the peak concentration was 10,025 spores/ml, at 750 m 2,340 spores/ml and at 1250 m 780 spores/ml. Spore counts above 30 spores/ml were obtained for 18 min at 150 m, for 29 min at 750 m but only at one time at 1250 m. The time to maximum count at 150 m was 9 min, at 750 m 18 min, and at 1250, 15 min.

### *Impact on Black Fly Larvae*

Mortality of black fly larvae achieved in the three treatments in the Carrabassett River is shown in Tables 2, 3, 4 and in No Name Stream in Table 5. In the June treatment in the Carrabassett River of 10 ppm for 1 min, a maximum mortality of 68% was attained 300 m below the application site (Table 2). In the July treatment at 10 ppm for 5 min, however, 98 - 100% mortality was attained up to 450 m below the application site (Table 3). Results from the August application at the same rate showed a similar trend with a 100% mortality at 150 m and 85.6% mortality at 750 m (Table 4). In No Name Stream

93.5 - 100% mortality was achieved at 10 ppm for 5 min up to 150 m below the application site (Table 5).

In the Carrabassett River, both the June 17 and July 11 applications of *B.t.i.* resulted in an immediate and substantial (8 and 20X respectively) increase in drift of black fly larvae (Fig. 2 and 3). Drift increased within 2 h of the *B.t.i.* application and had returned to pretreatment levels by the following morning. The most prevalent species in both June and July were *S. tuberosum*, *S. corbis* and *S. venustum* (Fig. 4 and 5). Larvae in the drift in the treated area immediately following treatment were dead and many showed signs of tissue breakdown. Black fly larvae were rare in the drift in No Name Stream both before and after the *B.t.i.* application. No data are presented for this stream.

#### *Impact on non-target invertebrates*

There was no evidence of a decrease in numbers of any of the taxa encountered in the substrates which could be attributed to the *B.t.i.* applications (Tables 6, 7, and 8). Increases or decreases in numbers in the treated areas which are paralleled by increases or decreases in the control areas must be attributed to factors such as recruitment or adult emergence rather than the *B.t.i.* application.

Taxa other than black fly larvae frequently encountered in the drift were Ephemeroptera, Chironomidae, Blepharoceridae and Acarina. There was no evidence of increased drift of any of these organisms following *B.t.i.* applications in the Carrabassett (Fig. 2 and 3). Organisms were rare in the drift in No Name Stream both before and after the *B.t.i.* application. No data are presented for this stream.

#### *Impact on Fish Feeding*

Analysis of the gut contents of slimy sculpins in No Name Stream and the Carrabassett River is shown in Tables 9 and 10. In No Name Stream (Table 9), out of 32 fish examined, only one black fly larva was found. Of a total of 39 fish collected in the Carrabassett River (Table 10), only five contained black fly larvae, and these contained only one black fly larva each. The mean percent of the total diet represented by black fly larvae ranged from 0-1.7%. Sculpins appeared to rely heavily on aquatic insects other than black fly larvae. In the Carrabassett River, mayflies, and in No Name Stream, chironomid larvae, were especially important.

Analyses of gut content of brook trout in No Name Stream and the Carrabassett River are shown in Tables 11, 12, and 13. In No Name Stream (Table 11) four of the 33 fish sampled contained one black fly larva each. There were two size classes of brook trout in the Carrabassett River: fry about 3 cm long and adult fish about 7 cm and longer. These two groups were examined separately. In the fry, (Table 12) six out of 12 fish contained black fly larvae, and

the mean number per fish on sampling dates ranged from 0–3.3. Feeding on black fly larvae in the control area appeared to be heavier than in the treated area. In the larger fish, (Table 13) 11 out of 28 fish contained black fly larvae, and the mean number per sample ranged from 0–1.1. There was a slight increase in numbers of black fly larvae ingested between pre- and posttreatment dates in both the control and treated areas, and it was not possible to attribute any change to the *B.t.i.* treatment. Trout in both the Carrabassett and No Name Stream fed extensively on terrestrial insects that had fallen into or onto the water. These terrestrial insects were abundant in the drift samples.

Data on the length and weight of fishes collected in the Carrabassett River and No Name Stream are included in Appendices ii and iii. Brook trout fry were abundant in the Carrabassett River but not in No Name Stream. The brook trout in both the Carrabassett River and No Name Stream were relatively small fish.

## DISCUSSION

In the Carrabassett River, *B.t.i.* proceeded down stream in the form of an initially high concentration, short duration slug which became progressively lower in concentration and longer in duration as the slug progressed down stream. Presumably spores were sedimented to the substrate, adsorbed to larger particles in the water column (large amounts of particulate organic matter were present in the drift), or caught in eddies. In any case, spores had almost disappeared from the water column 1250 m downstream from the application site.

*S. tuberosum*, *S. corbis* and *S. venustum* were the dominant species of black flies present in the Carrabassett River in June and July in 1985. Ninety percent mortality of black fly larvae is considered a satisfactory level of control (D. P. Molloy, personal communication). This level of control was not attained at any distance below the application site at the rate of 10 ppm for 1 min in the Carrabassett River. *B.t.i.* at the rate of 10 ppm for 5 min produced a satisfactory level of control for approximately 500 m below the application site in the Carrabassett River. Maximum mortality was attained within 2–4 h and did not increase at 24 h. Control in No Name Stream at the same application rate was less efficient, with satisfactory control extending only 150 m below the application site. This can be accounted for by the lower discharge in No Name Stream and the presence of pools; portions of the stream where flow is negligible and where the *B.t.i.* may drop out of the water column.

Rapid action of the *B.t.i.* is shown by the immediate and substantial increased drift of black fly larvae following application and by high mortality



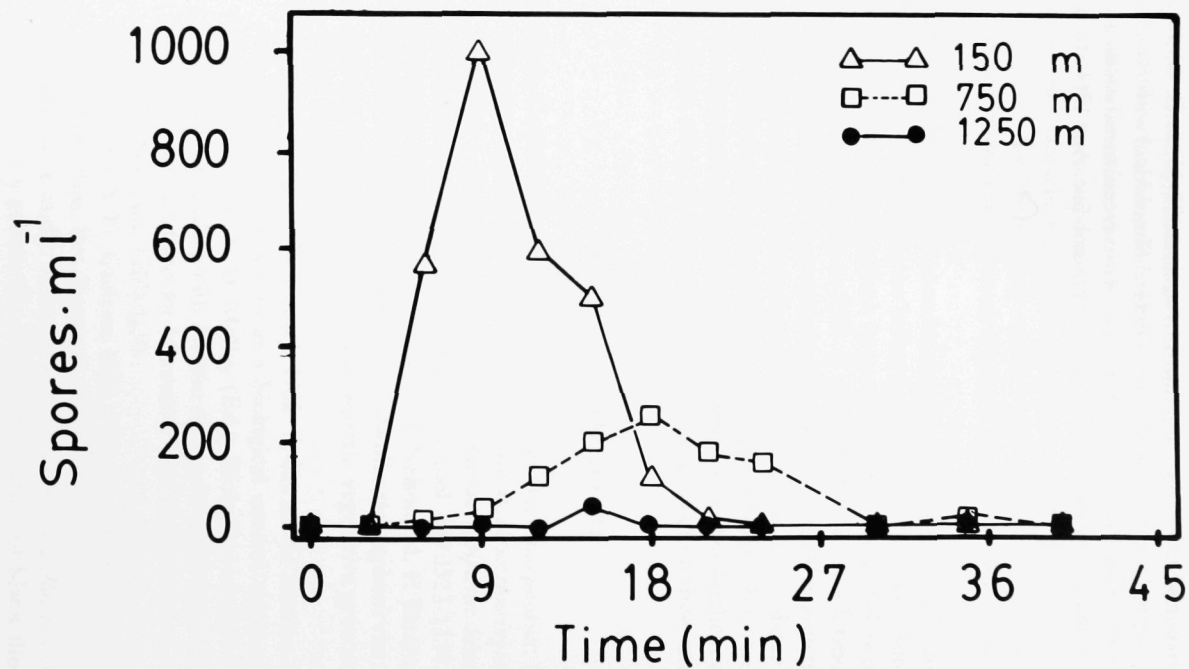
of larvae on the substrate within as little as 2 h. Large numbers of black fly larvae drifting were not considered in the living and dead counts. Thus the percent mortality in the treated areas of the Carrabassett River was probably higher than was recorded. There was no evidence of impact on non-target organisms in either the substrate or in increased drift. Increased drift had previously been reported for Trichoptera and Ephemeroptera (5) at an application rate which was the same as the maximum used in this study. The application rate in the study which reported the increased rate of drift in Blepharoceridae (1) was much higher  $5.28 \text{ g(LS)}^{-1}$  than that used in this study.

Slimy sculpins rarely fed on black fly larvae in either No Name Stream or the Carrabassett River either before or after *B.t.i.* treatment. Similarly, adult brook trout rarely fed on black fly larvae in either stream before or after *B.t.i.* treatment. However, up to 25% of the diet of small brook trout fry in the Carrabassett River was comprised of black fly larvae. Although these data do not indicate a change in numbers of black fly larvae ingested due to the *B.t.i.* treatment in this experiment this group of fish appears to be more dependent on these larvae than do other fish in the study area. Any further evaluations of non-target effects of *B.t.i.* applications should focus on these small fish.

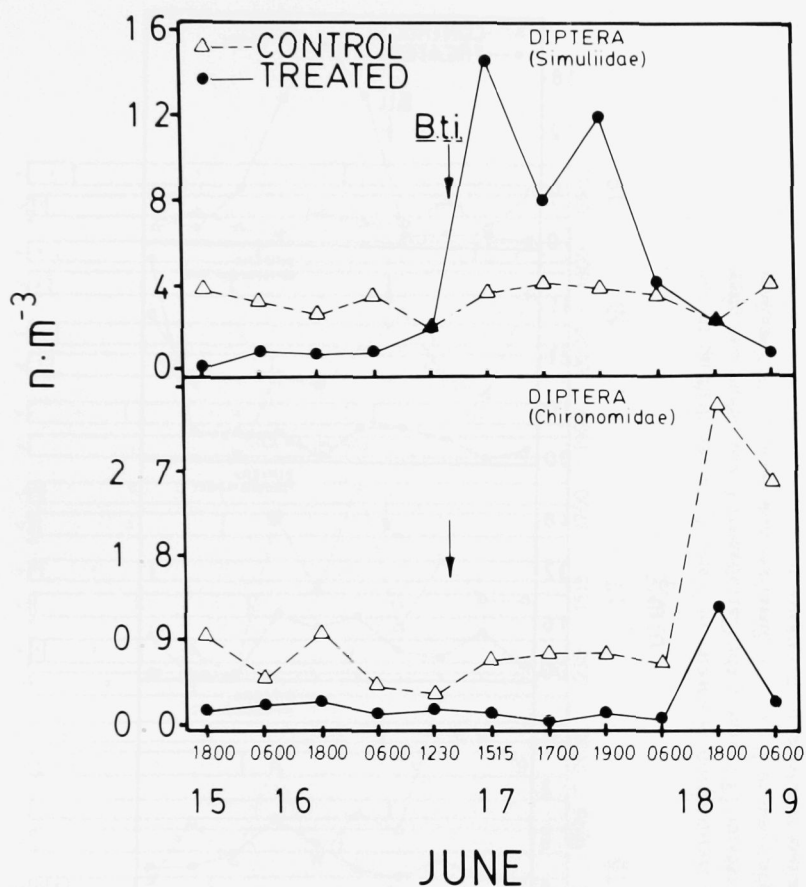
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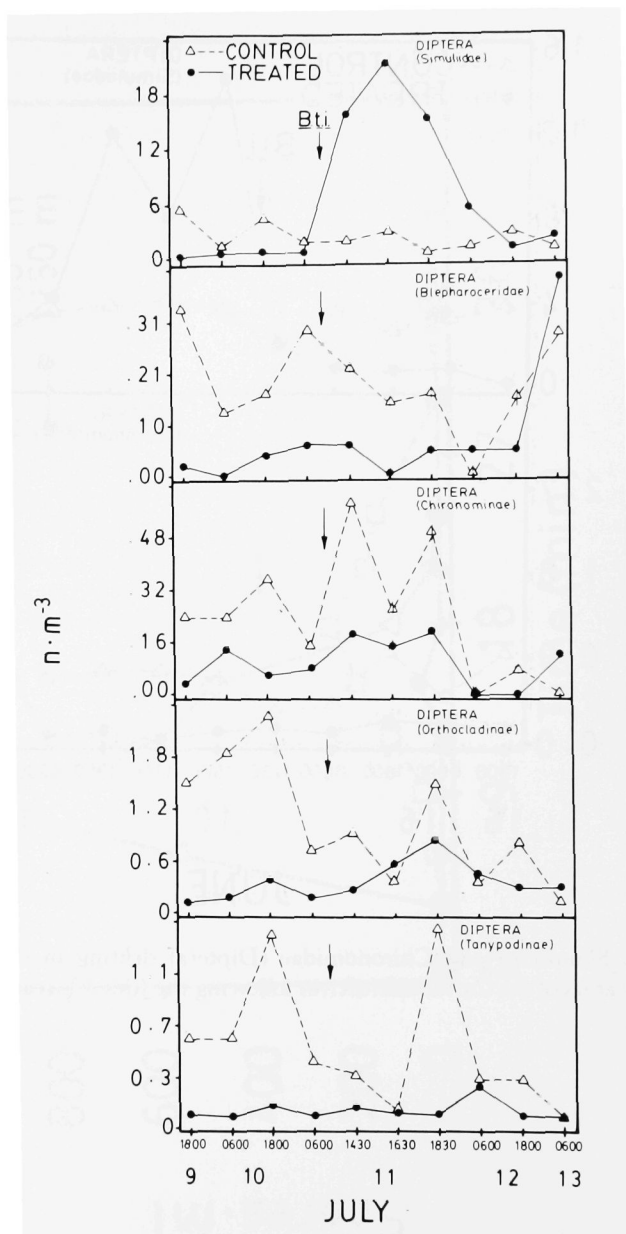
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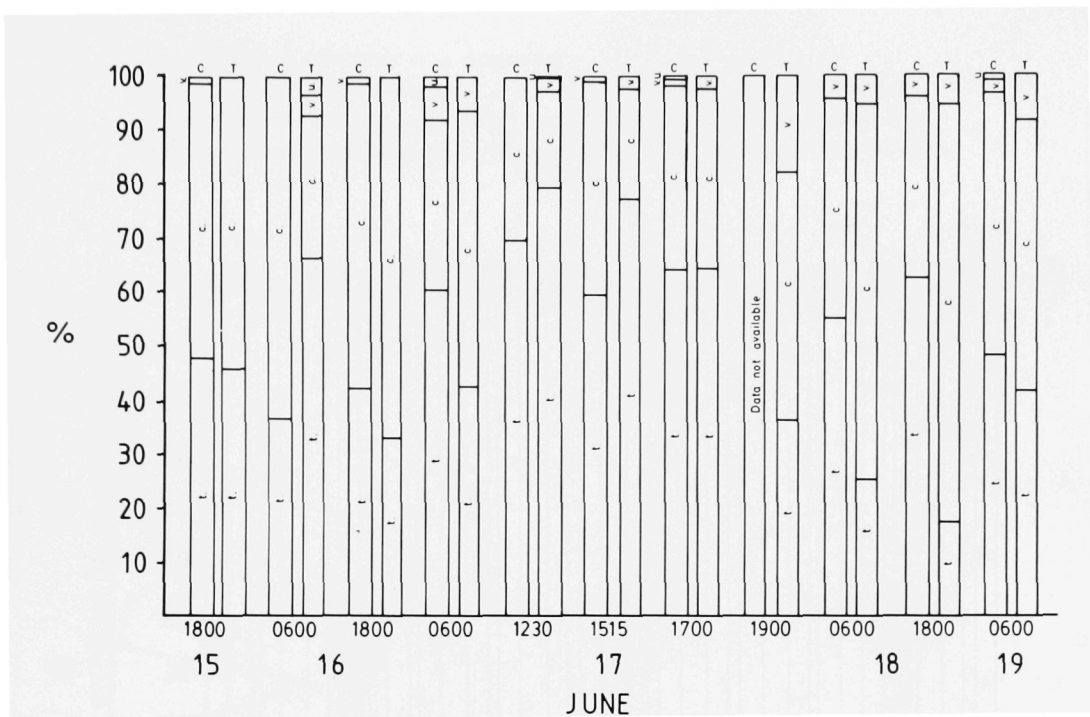
**Fig. 1.** The distribution of spores at 150 m, 750 m and 1250 m below the *B.t.t.* treatment site.



**Fig. 2.** Simuliidae and Chironomidae (Diptera) drifting in treated and control areas of the Carrabassett River following the June 17 treatment with *B.t.i.*



**Fig. 3.** Simuliidae, Blepharoceridae and Chironomidae (Diptera) drifting in treated and control areas of the Carrabassett River following the July 11 treatment with *B.t.i.*



**Fig. 4.** Percent composition of species of black fly larvae drifting in the control (C) and treated (T) areas of the Carrabassett River before and after the June 17 application of B.t.i. t. = *Simulium tuberosum*, c. = *Simulium corbis*. v. = *Simulium venustum*, u. = unknown.

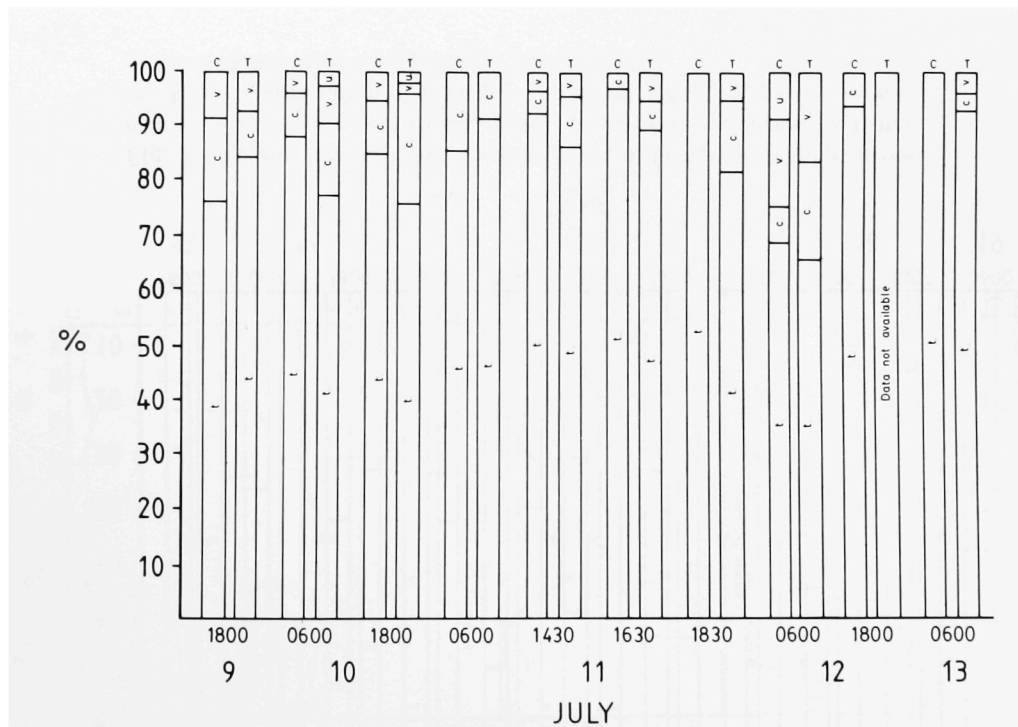


Fig. 5. Percent composition of species of black fly larvae drifting in the control (C) and treated (T) areas of the Carrabassett River before and after the July 11 application of *B.t.i.* t. = *Simulium tuberosum*, c = *Simulium corbis*, v. = *Simulium venustum*, u = unknown.

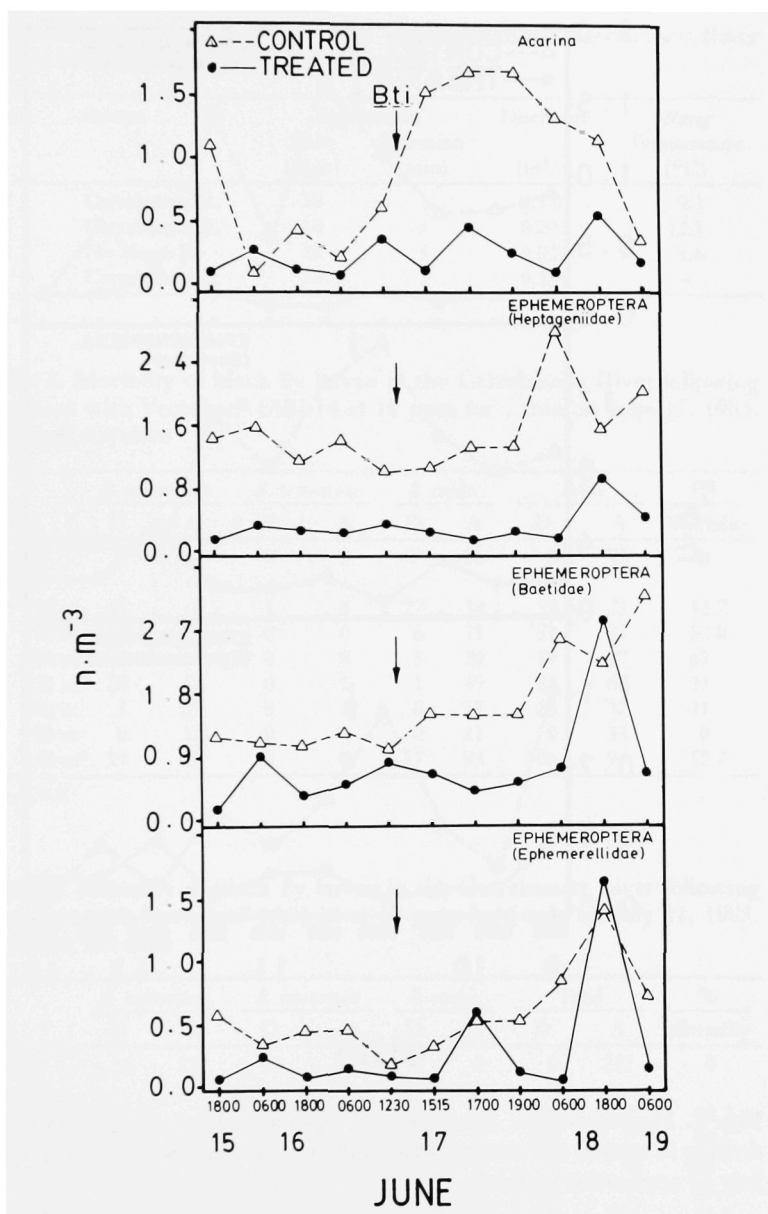
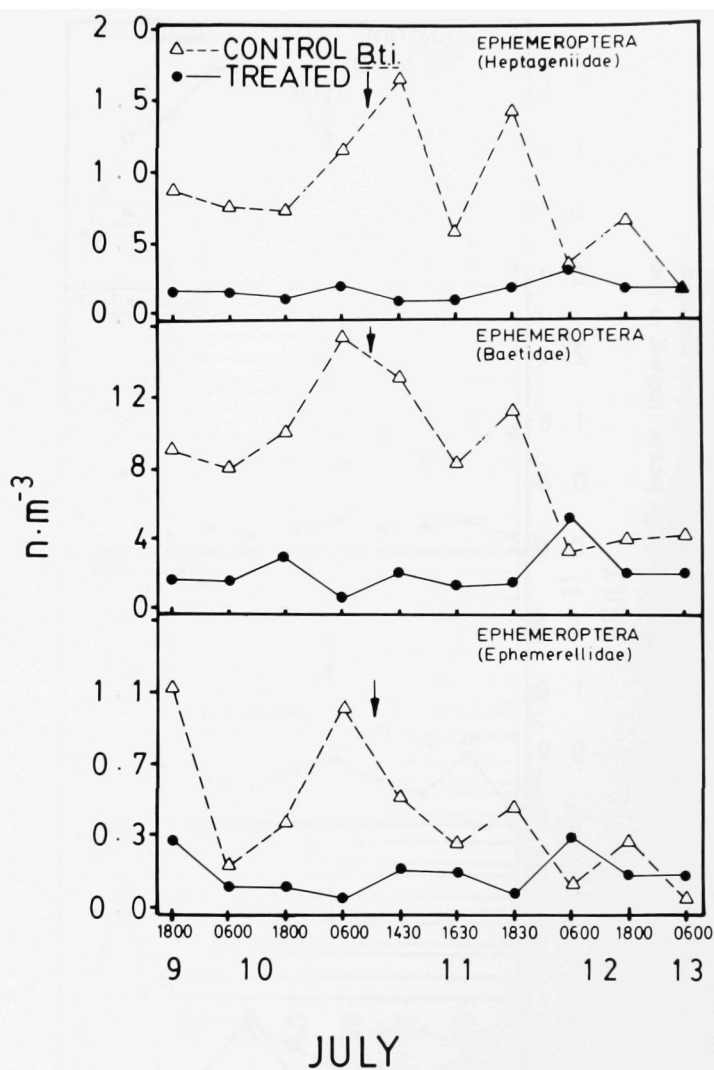


Fig. 6. Acarina and Heptageniidae, Baetidae and Ephemerellidae (Ephemeroptera) drifting in treated and control areas of the Carrabassett River following the June 17 treatment with *B.t.i.*





**Fig. 7.** Heptageniidae, Baetidae and Ephemerellidae (Ephemeroptera) drifting in treated and control areas of the Carrabassett River following the July 11 application of *B.t.i.*

**Table 1.** Experimental applications of Vectobac<sup>R</sup> to the Carrabassett River and No Name Stream during 1985.

Date	Stream	Application		Discharge (m <sup>3</sup> /s)	Water Temperature (°C)
		Rate (ppm)	Duration (min)		
6/17	Carrabassett R.	10	1	0.156	9.3
7/11	Carrabassett R.	10	5	0.205	12.1
7/11	No Name St.	10	5	0.027	9.6
8/5	Carrabassett R.	10	5	0.154	—

**Table 2.** Mortality of black fly larvae in the Carrabassett River following treatment with Vectobac<sup>R</sup> (AS)-14 at 10 ppm for 1 min on June 17, 1985. D=dead; A=alive

Site	<i>S. tuberosum</i>		<i>S. venustum</i>		<i>S. corbis</i>		Total		%
	D	A	D	A	D	A	D	A	Mortality
Control	0	12	0	2	0	76	0	95	0
Treated									
50 m	11	0	1	0	27	34	39	35	52.7
150 m	44	0	0	0	6	35	51	35	60.0
300 m	51	7	0	0	5	20	59	27	68
450 m	20	17	0	1	1	49	21	67	23
600 m	5	29	0	1	0	17	23	47	31
750 m	0	13	0	0	0	21	0	33	0
50 m*	34	2	0	0	57	93	106	95	52.7

\*after 24 h

**Table 3.** Mortality of black fly larvae in the Carrabassett River following treatment with Vectobac<sup>R</sup> (AS)-14 at 10 ppm for 5 min on July 11, 1985. D=dead; A=alive

Site	<i>S. tuberosum</i>		<i>S. venustum</i>		<i>S. corbis</i>		Total		%
	D	A	D	A	D	A	D	A	Mortality
Control	0	222	0	0	0	0	0	222	0
Treated									
50 m	141	2	2	0	7	0	150	2	98.7
150 m	549	2	12	0	38	0	599	2	99.6
300 m	19	0	0	0	0	0	19	0	100
450 m	167	1	8	0	3	0	179	1	99.4
600 m	53	37	1	0	3	44	57	81	41.3
750 m	41	22	1	0	1	2	47	24	66
900 m	11	13	0	0	0	0	11	13	45
1050 m	0	23	0	1	0	0	0	24	0

**Table 4.** Mortality of black fly larvae in the Carrabassett River following treatment with Vectobac<sup>R</sup> (AS)-14 at 10 ppm for 5 min on August 5, 1985. D=dead; A=alive

Site	<i>S. tuberosum</i>		<i>S. venustum</i>		<i>S. corbis</i>		Total		%
	D	A	D	A	D	A	D	A	Mortality
150 m	83	0	10	0	5	0	98	0	100
750 m	143	24	0	0	0	0	143	24	85.6
1250 m	65	117	0	0	0	0	65	117	35.1

**Table 5.** Mortality of black fly larvae in the Carrabassett River following treatment with Vectobac<sup>R</sup> (AS)-14 at 10 ppm for 5 min on July 11, 1985. D=dead; A=alive

Site	<i>S. tuberosum</i>		<i>S. venustum</i>		<i>S. corbis</i>		Total		%
	D	A	D	A	D	A	D	A	Mortality
Control	0	6	0	59	0	0	0	65	0
Treated									
14 m	16	0	40	0	0	0	58	0	100
50 m	20	1	67	0	2	0	89	1	98.8
100 m	29	0	0	3	0	0	29	3	93.5
150 m	16	0	2	0	1	0	19	0	100
200 m	2	4	18	10	0	0	20	4	83
250 m	6	33	0	14	0	0	20	33	37.7
300 m	0	6	0	19	0	0	0	25	0
350 m	0	8	0	20	0	0	0	28	0

**Table 6.** Mean numbers of macroinvertebrates in samples from control and treated areas of the Carrabassett River before and after the June 17 treatment with *B.t.i.*

Date	Control		Treated	
	Pretreat 6/14	Post-treat 6/18	Pretreat 6/14	Post-treat 6/18
Taxa				
Ephemeroptera				
Ephemerellidae	19	23.4	15.3	49.2
Heptageniidae	4	20.8	7.3	25.0
Siphonuridae	1	3.9	1.7	7.3
Plecoptera				
Perlodidae	0.3	1.6	1.3	2.5
Trichoptera				
Lepidostomidae	6	2.5	0.3	2.3
Diptera				
Blepharoceridae	1.3	0.4	0	0.5
Athericidae	0.7	1.3	0.3	1.8
Chironomidae	24	25.5	12	59.7
Simuliidae				
<i>S. venustum</i>	0.3	4.8	3.3	9.3
<i>S. corbis</i>	62.3	3.5	110.3	4.3
<i>S. tuberosum</i>	33	5.0	46.7	12.5
Coleoptera				
Elmidae	3	3.8	1.3	3.0
Acarina	0.3	1.5	0.3	2.0

**Table 7.** Mean numbers of macroinvertebrates in samples from control and treated areas of the Carrabassett River before and after the July 11 treatment with *B.t.i.*

Date	Control		Treated	
	Pretreat 7/10	Post-treat 7/15	Pretreat 7/10	Post-treat 7/15
Taxa				
Ephemeroptera				
Ephemerellidae	0.8	2.8	7	7.8
Ephemeridae	0	3.5	0	2.4
Heptageniidae	19	21.0	10.5	26.4
Siphonuridae	26	13.0	26.5	3.6
Plecoptera				
Perlodidae	36.5	15.5	17.3	8.8
Trichoptera				
Lepidostomidae	1.3	3.3	1.5	1.6
Diptera				
Athericidae	4	4	3.3	3
Chironomidae	569.7	289.5	187.25	108.25
Simuliidae				
<i>S. venustum</i>	2.25	2.8	2.5	3.0
<i>S. corbis</i>	0	0	0	0
<i>S. tuberosum</i>	3.8	2.3	5.5	8.8
Coleoptera				
Elmidae	5.3	9.3	0.3	3.6
Acarina	7.5	1.8	2.5	1.2

**Table 8.** Mean numbers of macroinvertebrates in samples from control and treated areas of the No Name Stream before and after the July 11 treatment with *B.t.i.*

Date	Control		Treated	
	Pretreat 7/11	Post-treat 7/15	Pretreat 7/11	Post-treat 7/15
Taxa				
Ephemeroptera				
Ephemerellidae	0.3	2	1	1.5
Heptageniidae	1.0	1	0	0
Siphonuridae	4.3	4	1.7	1.5
Plecoptera				
Perlodidae	37.7	23.5	27	13
Trichoptera				
Lepidostomidae	3.3	6	0.3	1
Diptera				
Athericidae	7.7	3.5	6.0	2.5
Chironomidae	306.5	237.0	365.0	153.0
Simuliidae				
<i>S. venustum</i>	0.3	0	0.3	0.5
<i>S. corbis</i>	0	0	0	0
<i>S. tuberosum</i>	0	0	0	0
Coleoptera				
Elmidae	1	0.5	3.3	0
Acarina	3.3	3.5	5.3	1.5

**Table 9.** Diet of slimy sculpins *Cottus cognatus* in No Name Stream before and after treatment with 10 ppm of *B.t.i.* for 5 min on July 11, 1985. P/T=number of fish in which a food category is present/total number of fish in the sample;  $\bar{x}n$ =mean number of organisms per fish;  $\bar{x}\%$ =mean percent of food category in the gut content; Sim.=Simuliidae larvae; Bleph.=Blepharoceridae larvae; O. Diptera=other Diptera larvae; Diptera P.=Diptera pupae; Ephem.=Ephemeroptera nymphs; Plec.=Plecoptera nymphs; Tric.=Trichoptera larvae; Terr.=terrestrial invertebrates; Misc.=miscellaneous unidentified material.

	Date	Sim.				Bleph.		O. Diptera		Diptera P.		Ephem.		Plec.		Tric.		Terr.		Misc.
		P/T	$\bar{x}$ n	$\bar{x}$ %		P/T	$\bar{x}$ %	P/T	$\bar{x}$ %	P/T	$\bar{x}$ %	P/T	$\bar{x}$ %	P/T	$\bar{x}$ %	P/T	$\bar{x}$ %	P/T	$\bar{x}$ %	
Pretreatment																				
Control	7/9	0/10	0	0	0/10	0	7/10	14.1	0	0	2/10	6.6	2/10	3.1	5/10	6.6	1/10	0.7	66.3	
Treated	7/9	0/9	0	0	0/9	0	3/9	7.3	1/9	0.4	0/9	0	1/9	0.3	0/9	0	1/9	1.9	74.6	
Post-treatment																				
Control	7/15	1/10	0.1	0.7	0/10	0	5/10	2.9	0/10	0	2/10	5.9	0/9	0	5/9	2.9	1/9	0.7	74.6	
Treated	7/15	0/3	0	0	0/3	0	2/3	12.0	1/3	7.1	0/3	0	0/3	0	2/3	12.0	6/3	0	57.5	

**Table 10.** Diet of slimy sculpins (*Cottus cognatus*) in the Carrabassett River before and after treatment with 10 ppm of *B.t.i.* for 1 min on June 17, 1985. Conventions as in Table 9.

	Date	Sim.		Bleph.		O. Diptera		Diptera P.		Ephem.		Plec.		Tric.		Terr.		Misc.	
		P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	x%	
Pretreatment																			
Control	6/15	1/10	0.1	0.1	3/10	5.1	4/10	1.7	1/10	1.0	6/10	26.5	0	0	3/10	1.8	1	1.3	61.6
Treated	6/15	0/10	0	0	2	1.9	1/10	0.4	0	0	10/10	58.1	1/10	1.7	1/10	3.1	0	0	34.7
Post-treatment																			
Control	6/20	1/9	0.2	.6	3/9	2.3	6/9	6.6	2/9	0.4	7/9	11.7	2/9	3.2	4/9	3.0	0	0	71.7
Treated	6/19	3/10	0.3	1.7	2/10	2.1	5/10	3.6	0	0	6/10	17.6	0	0	3/10	4.9	1/10	.08	69.6

**Table 11.** Diet of adult brook trout (*Salvelinus fontinalis*) in No Name Stream before and after treatment with 10 ppm of *B.t.i.* for 5 min on July 11, 1985. Conventions as in Table 9.

	Date	Sim.				Bleph.		O. Diptera		Diptera P.		Ephem.		Plec.		Tric.		Terr.		Misc.
		P/T	n	x%	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	x%
Pretreatment																				
Control	7/9	2/9	0.2	0.3	0/9	0	5/9	2.5	5/9	3.6	4/9	2.7	4/9	2.8	6/9	12.0	8/9	16.4		60.5
Treated	7/9	2/9	0.2	0.3	0/9	0	3/9	1.9	1/9	0.2	2/9	2.4	2/9	0.6	5/9	3.2	8/9	27.8		63.7
Post-treatment																				
Control	7/15	0/10	0	0	0/10	0	5/10	0.9	1/10	0.6	3/10	0.8	0/10	0	3/10	1.9	10/10	10.9		84.8
Treated	7/15	0/5	0	0	0/5	0	3/5	4.5	5/5	6.7	2/5	9.3	0/5	0	3/5	6.7	4/5	12.3		65.1



**Table 12.** Diet of brook trout fry (*Salvelinus fontinalis*) in the Carrabassett River before and after treatment with 10 ppm of *B.t.i.* for 1 min on June 17, 1985. Conventions as in table 9.

Date	Sim.				Bleph.		O. Diptera		Diptera P.		Ephem.		Plec.		Tric.		Terr.		Misc.
	P/T	xn	x%		P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	x%
Pretreatment																			
Control	6/15	2/3	1.3	25.3	0	0	3/3	11.3	0/3	0	3/3	21.9	0	0	0	0	2/3	13.1	27.4
Treated	6/15	0/3	0	0	0	0	3/3	10.8	0/3	0	2/3	5.3	1	1.4	1	1.4	1/3	5.8	71.8
Post-treatment																			
Control	6/19	2/3	3.3	7.5	1	1.6	2/3	6	1	0.3	3/3	5.8	0/3	0	3	7.3	3/3	10.2	65.3
Treated	6/19	2/3	1.3	5.0	0	0	2/3	18.2	0	0	1/3	12.1	1/3	6.1	1/3	1	3/3	11.9	45.2

**Table 13.** Diet of adult brook trout (*Salvelinus fontinalis*) in the Carrabassett River before and after treatment with 10 ppm with *B.t.i.* for 1 min on June 17, 1985. Conventions as in Table 9.

Date	Sim.				Bleph.		O. Diptera		Diptera P.		Ephem.		Plec.		Tric.		Terr.		Misc.
	P/T	xn	x%		P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	P/T	x%	x%
Pretreatment																			
Control	6/15	0	0	0	1/7	0.7	0	0	2/7	0.3	2/7	1.2	1/7	0.8	3/7	0.9	6/7	23.8	61.1
Treated	6/15	2/7	0.7	0.2	2/7	0.8	3/7	0.7	3/7	0.4	4/7	6.5	5/7	2.1	4/7	2.1	7/7	26.3	62.8
Post-treatment																			
Control	6/19	4/7	1.1	2.5	3/7	1.4	3/7	3.7	0	0	7/7	20.8	2/7	1.2	5/7	4.5	6/7	10.0	54.8
Treated	6/19	5/7	1.0	1.0	3/7	0.9	6/7	3.5	3/7	1.0	6/7	10.5	2/7	0.5	4/7	3.4	6/7	21.5	57.4

**Appendix i.** Length and weight of fishes collected in the Carrabassett River.

Location	Species	Date	Number of Fish	Length (cm)		Weight (g)	
				$\bar{x}$	Range	$\bar{x}$	Range
Treated	Brook trout (fry)	6/15	3	3	3	.25	.25
		6/19	3	3.5	3.5	.4	.4
	Brook trout (juvenile)	6/15	7	9.7	7.0-13.5	11.4	3.4-24.4
		6/19	7	8.1	6.8-9.3	6.7	4.3-8.5
	Slimy sculpin	6/15	10	6.0	3.3-7.9	2.8	0.7-5.3
		6/19	10	5.9	4.3-7.8	2.6	1.1-5.6
Control	Brook trout (fry)	6/15	3	3	3	.25	.25
		6/19	3	3.5	3.5	.4	.4
	Brook trout (juvenile)	6/15	7	11.2	8.4-19.0	19.3	5.9-17.5
		6/19	7	9.9	7.7-14.2	11.4	4.5-29.6
	Slimy sculpin	6/15	10	6.4	3.8-8.1	3.3	0.5-5.2
		6/19	10	5.8	4.2-7.7	2.6	1.0-5.6

**Appendix ii.** Length and weight of fishes collected in No Name Stream.

Location	Species	Date	Number of Fish	Length (cm)		Weight (g)	
				$\bar{x}$	Range	$\bar{x}$	Range
Treated	Brook trout	7/9	10	6.7	4.0-11.8	7.1	.5-18.7
		7/15	5	4.0	4	.8	.8
	Slimy sculpin	7/9	9	4.5	3.2-6.5	1.5	.4-2.9
		7/15	3	3.9	3.9	.8	.8
Control	Brook trout	7/9	9	8.3	4.4-14.3	7.8	.1-33.9
		7/15	10	9.0	4.0-17.8	15.2	.75-68.2
	Slimy sculpin	7/9	10	5.2	3.5-6.5	1.5	.4-2.17
		7/15	10	5.0	3.3-7.0	1.8	.5-4.1